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(54) Rapid detection of nucleic acid sequences in a sample by labeling the sample.

(57) A method for detecting one or more microorganisms or polynucleotide sequences from eukaroytic sources in a nucleic acid-containing test sample comprising

(a) preparing a test sample comprising labeling the nucleic acids in the test sample,

(b) preparing one or more process by immobilizing a single-stranded nucleic acid of one or more known microorganisms or sequences from eukaroytic sources,

(c) contacting, under hybridization conditions, the labeled single-stranded nucleic acid to form hybridized labeled nucleic acids, and

(d) assaying for the hybridized nucleic acids by detecting the label. The method can be used to detect genetic disorders, e.g., sickle-cell anemia.

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RAPID DETECTION OF NUCLEIC ACID SEQUENCES IN A SAMPLE BY LABELING THE SAMPLE

BACKGROUND OF THE INVENTION

Field of the Invention

The present application relates to the detection and identification of microorganisms and the detection and identification of particular prokaryotic or eukaryotic DNA sources in a nucleic acid containing test sample.

Still further, the present invention relates to a method for the lysis of whole cells.

Background Information

A. The Detection of Microorganisms

The identification of species of microorganisms in a sample containing a mixture of microorganisms, by immobilizing the DNA from the sample and subjecting it to hybridization with a labelled specimen of species -specific DNA from a known microorganism and observing whether hybridization occurs between the immobilized DNA and the labelled specimen, has been disclosed in PCT patent application No. PCT/US83/01029.

The most efficient and sensitive method of detection of nucleic acids such as DNA after hybridization requires radioactively labeled DNA. The use of autoradiography and enzymes prolongs the assay time and requires experienced technicians.

U.S.P. 4,358,535 to Falkow et al describe infectious disease diagnosis using labeled nucleotide probes complementary to nucleic acid coding for a characteristic pathogen product.

B. The Detection of Specific Eukaryotic Sequences

The identification of specific sequence alteration in an eukaryotic nucleic acid sample by immobilizing the DNA from the sample and subjecting it to hybridization with a labeled oligonucleotide and observing whether hybridization occurs between the immobilized DNA and the labeled probe, has been described in EP -patent application No. 86 117 978 filed December 23, 1986, now pending.

It is known that the expression of a specific gene determines the physical condition of a human being. For example, a change in the beta-globin gene coding sequence from GAG to GTG at the sixth amino acid position produces sickle-beta-globin and a homozygote can have a disease known as sickle cell anemia. Similarly deletion of particular sequences from alpha-globin or beta-globin genes can cause thalassemias. A recent survey, The New Genetics and Clinical Practice, D.J. Weatherall, The Nuffield Provincial Hospitals Trust, (1982), chapter 2 describes the frequency and clinical spectrum of genetic diseases.

Problems associated with genetic defects can be diagnosed by nucleic acid sequence information. The easiest way to detect such sequence information is to use the method of hybridization with a specific probe of a known sequence.

U.S.P. 4,395,486 to Wilson et al describe a method for the direct analysis of sickle cell anemia using a restriction endonuclease assay.

Edward M. Rubin and Yuet Wai Kan, "A Simple Sensitive Prenatal Test for Hydrops Fetalis Caused By α-Thalassaemia", The Lancet, January 12, 1985, pp. 75-77 describes a dot blot analysis to differentiate between the genotypes of homozygous alpha-thalassemia and those of the haemoglobin-H disease and alpha-thalassemia trait.

The most efficient and sensitive method of detection of nucleic acids, such as DNA, after hybridization requires radioactively labelled DNA. The use of autoradiography and enzymes prolongs the assay time and requires experienced technicians.

Recently, a non-radioactive method of labelling DNA was described by Ward et al, European Patent Application 63,879. Ward et al, use the method of nick translation to introduce biotinylated U (uracil) residues into DNA, replacing T (thymine). The biotin residue is then assayed with antibiotin antibody or an avidin-containing system. The detection in this case is quicker than autoradiography, but the nick translation

method requires highly skilled personnel. Moreover, biotinylation using biotinylated UTP (uridine triphosphate) works only for thymine-containing polynucleotides. The use of other nucleoside triphosphates is very difficult because the chemical derivatization of A (adenine) or G (guanine) or C (cytosine) (containing $-NH_2$) with biotin requires the skills of trained organic chemists.

C. Cell Lysis

The present invention also provides a method for the efficient lysis of whole cells such that their DNA is released and made available for photochemical labeling. While eukaryotic cells derived from multicellular animals are easily lysed under relatively mild conditions, single cell eukaryotes and prokaryotes, especially Gram positive prokaryotes, are more difficult to lyse due to the complicated chemical nature and extent of cross-linking of their cell walls. Methods do exist for efficiently lysing these refractory organisms, either by chemical-enzymatic or physical means, but these methods are often complicated, time-consuming and inappropriate for preserving the integrity of DNA.

SUMMARY OF THE INVENTION

It is accordingly an object of the present invention to provide a method for detection of microorganisms in a nucleic acid-containing test sample.

It is another object of the invention to provide a method for a simultaneous assay for the presence of more than one nucleic acid sequence.

Another object is to provide a method to identify particular prokaryotic or eukaryotic DNA sequences and a method for distinguishing alleles of individual genes.

Another object of the invention is to provide a simple photochemical method of labeling the unknown test sample.

A further object of the invention is to label the probes with different kinds of labels so that when the probes are hybridized with an immobilized, unknown, unlabelled test sample, the type of label remaining bound after hybridization and washing, will determine the type of nucleic acid sequence present in the unknown sample.

A still further object of the invention is to use whole chromosomal nucleic acid as the probe and/or as the test sample.

Also the invention relates to the use of oligonucleotides as immobilized probes.

These and other objects and advantages are realized in accordance with the present invention for a method of detecting nucleic acid sequences in a nucleic acid-containing test sample.

The method involves the following:

(a) preparing a test sample comprising labeling the nucleic acids of the organisms or cells or cell debris in the test sample,

(b) preparing one or more probes by immobilizing a single-stranded DNA or an oligonucleotide of one or more known microorganisms or eukaryotes, or sequences representing particular genes or their alleles,

(c) contacting, under hybridization conditions, the labeled single-stranded sample nucleic acid and the immobilized single-stranded (probe) nucleic acid or the immobilized oligonucleotide to form hybridized labeled nucleic acids and

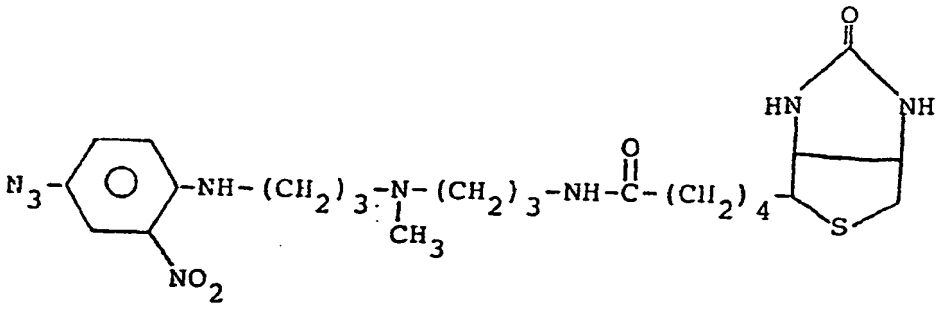
(d) assaying for the hybridized nucleic acids by detecting the label.

In the above method, steps (a) and (b) can be reversed.

The method further comprises denaturing the labeled nucleic acids from step (a) to form labeled denatured nucleic acids.

According to the invention, a labeled nucleic acid test sample is contacted simultaneously with several different types of DNA probes for hybridization. The nucleic acid test sample is labeled and hybridized with several unlabeled immobilized probes. The positions of the probes are fixed, and the labeled probe detected after hybridization will indicate that the test sample carries a nucleic acid sequence complementary to the corresponding probe.

Nucleic acid probes for several microbiological systems or for different alleles of one or more genes can be immobilized separately on a solid support, for example, nitrocellulose paper. The test sample nucleic acids are labeled and remain in solution. The solid material containing the immobilized probe is brought in contact with the labeled test nucleic acid solution under hybridization conditions. The solid



Other useful photoreactable intercalators are the furocoumarins which form (2+2) cycloadducts with pyrimidine residues. Alkylating agents can also be used such as bis-chloroethylamines and epoxides or aziridines, e.g., aflatoxins, polycyclic hydrocarbon epoxides, mitomycin and norphillin A.

Nonlimiting examples of intercalator compounds for use in the present invention include acridine dyes, phenanthridines, phenazines, furocoumarins, phenothiazines and quinolines.

The label which is linked to the nucleic acid component according to the present invention can be any chemical group or residue having a detectable physical or chemical property, i.e., labeling can be conducted by chemical reaction or physical adsorption. The label will bear a functional chemical group to enable it to be chemically linked to the intercalator compound. Such labeling materials have been well

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